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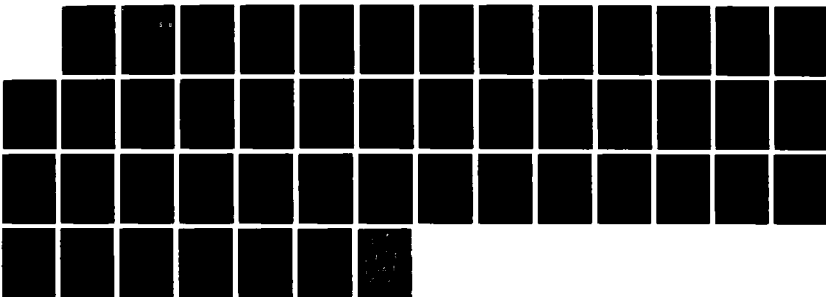
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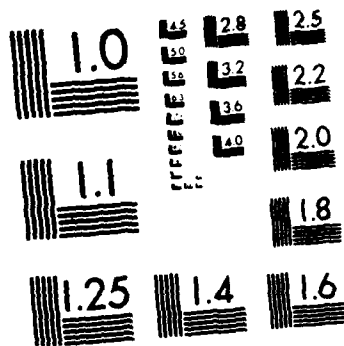
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ENZYME MINI-TEST FOR FIELD IDENTIFICATION OF  
LEISHMANIA ISOLATES FROM U.S. MILITARY  
PERSONNEL

Annual and Final Report

RICHARD D. KREUTZER, PH.D.

MAY 15, 1986

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701-5012

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Youngstown, Ohio 44555

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## REPORT DOCUMENTATION PAGE

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<p>➤ It is possible to identify <u>Leishmania</u> isolates by cellulose acetate electrophoresis (CAE) of up to 29 enzyme activities. Certain of these enzymes are polymorphic within a subspecies and therefore of limited value for identification; others are monomorphic and have taxonomic significance. Once large numbers of isolates from various geographical areas have been characterized and monomorphic enzymes identified, a simple, rapid, accurate field type identification test can be devised.</p> <p>Data for up to 29 enzymes have been obtained from about 400 <u>Leishmania</u> isolates by CAE. Among the isolates were two groups designated as reference strains. Enzyme profiles have been established for many <u>Leishmania</u> subspecies based on CAE data from over 20 widely distributed isolates and reference strains. The <u>Leishmania</u> profiles thus established are as follows: <u>L. braziliensis panamensis</u> (L.b.p. 104 isolates), <u>L. b. braziliensis</u> (L.b.b. - 56), <u>L. b. guyanensis</u> (L.b.g. - 28), <u>L. mexicana mexicana</u> / - <u>pifanoi</u> (L.m.m./p. - 43/2),</p>			
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L. m. amazonensis/garnhami (L.m.a./g. - 47/2), L. m. aristedesi (L.ar. - 1), L. m. enriettii (L.m.e. - 2), L. donovani (L.d.d. - 28), L. chagasi (L.d.c. - 27), L. major (L.m.j. - 32), L. tropica (L.t. - 25), L. aethiopica (L.ae. - 7), L. hertigi (L.h.h. - 4), L. gerbilli (L.ge. - 1). No consistent CAE differences have been noted between L.m.m. and L.m.p. or between L.m.a. and L.m.g.; therefore, they have been noted as L.m.m./p. and L.m.a./g. respectively.

As the number of isolates studied from a particular geographical area increased so did the diversity of Leishmania subspecies; therefore, to obtain an accurate estimate of the Leishmania fauna in an area many isolates must be identified. Routine CAE identification was performed for certain WRAIR contractees and other sources. When appropriate, data from these isolates were included in subspecies enzymes profiles. Isolates obtained by the P.I. in Colombia and identified by CAE have been sent to and have been added to the WRAIR cryobank. It was suggested that preliminary and confirmatory CAE identification be made a part of all WRAIR Leishmania studies. The enzyme profile data from 400 isolates suggest that accurate CAE identification can be made from data of GPI, MPI and 6PGDH. The buffer and the stain components for these enzymes can be prepackaged, and data can be obtained from  $10^6$  Leishmania cells.

The CAE data which have been generated in this and other studies have been compiled in a computer program. The data were recorded on a disk which was sent to the Leishmania Section at WRAIR and therefore are available to WRAIR personnel.

A detailed short test for Leishmania identification by CAE was included for the enzymes GPI, MPI, 6PGDH. The test included supplies and equipment with purchasing information needed for CAE, cell preparation for CAE procedures, complete set-up and running information, controls and confirming procedures, sequence of enzymes to study and diagrams of GPI, MPI and 6PGDH enzyme data. Most Leishmania could be identified from data obtained from this test.

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## Problem

Leishmania isolates from any source can be accurately and rapidly identified by cellulose acetate electrophoresis (CAE). Studies using 29 enzyme activities have shown that certain enzymes are polymorphic and of limited value in identification, while others are monomorphic and have taxonomic significance. It is, therefore, necessary to characterize isolates from a series of geographic areas by examining data from a number of enzymes to find a few monomorphic enzymes that can be used in a simple, rapid, accurate field identification test.

## Background

Electrophoretic study of gene-enzyme systems represents a powerful tool in systematics, genetics, ecology, ethology and applied biology. Studies on the systematic value of electrophoretic data reveal high levels of genetic similarity between conspecific populations, with up to 85% identity of their loci, while the percent of genetic similarities among closely related species are usually much lower.<sup>1-4</sup>

Recently enzyme electrophoresis has been used to identify parasites of the genus Leishmania<sup>5-11</sup>. One study reported that 21% of the enzymes tested from particular subspecies were polymorphic.<sup>11</sup> This suggests that isolates which have profiles greater than 75% identical are the same subspecies, and isolates which are less than 75% identical are parasites from different subspecies. The enzyme profiles of some isolates in this study were identical, but in most cases, the profiles among isolates in a given subspecies were

different. Leishmania could be grouped into at least five major complexes according to enzyme profiles, braziliensis, mexicana, donovani, tropica and hertigi. These designations for the most part are consistent with the taxonomic categories used by others.<sup>12</sup> Within these complexes the isolates could be grouped into subspecies with varying levels of allozyme identity. It has been reported that the L. braziliensis complex is composed of two possibly three (L. b. guyanensis) subspecies, L. b. panamensis and L. b. braziliensis. The L. mexicana complex has at least three subspecies, L. m. mexicana, L. m. amazonensis and L. m. aristidesi. Minimal numbers of L. tropica and L. donovani had been examined and subspecies remained to be delineated. These data were consistent with those obtained by the principal investigator in an earlier less comprehensive study. Results of this study demonstrated that electrophoresis can be used for rapid and accurate subspecific identification of Leishmania.<sup>11</sup>

The data which have been reported on enzyme profiles of leishmanial groups have been obtained from small numbers of isolates. Among known species there is a certain amount of naturally occurring enzyme polymorphism. Isolates which have been identified as belonging to the same group by classical methods and their identification confirmed by CAE do have slight genetic differences as noted from their individual enzyme profiles. This type of population polymorphism is to be expected and has been noted in all groups of organisms which have been studied by electrophoresis.<sup>1-4</sup>



A taxonomic enzyme profile must include as much information on polymorphism as can be obtained. The problems which can result from establishing taxonomic enzyme profiles from small numbers of isolates are noted in the following example. If L. donovani WR130 from Khartoum and L. donovani WR352 from India are compared, 68% of 25 enzymes would be identical by CAE, but when the WR352 profile is compared to ten other L. donovani isolates, the levels of identity are greater. Furthermore, if the enzyme analysis were confined to MDH, ICD, GOT, ALAT, AK1, EST, ACPl, MPI (Table 1) there would have been 0% identity and the two isolates would have been considered as two different species complexes. Therefore, taxonomic profiles must be based on many systems and on data from a large number of isolates representing the entire geographical distribution of the group.

The identification of Leishmania based on 29 genetic loci produces more accurate results; however, it should be possible to make a rapid and accurate identification using only 2 or 3 enzymes once the geographic area of isolation is known. For example the WRAIR isolates WR209 (L. b. panamensis), WR359 (L. b. braziliensis), WR225 (L. m. amazonensis), WR381 (L. m. mexicana), and WR285 (L. chagasi) are all from Panama. These isolates can be separated as follows:

GPI	209 and 359 are identical. This pair differs from 225, 381 and 285 which differ from one another.
ALAT	359 and 285 are identical. This pair differs from all others.
MPI	Each differs one from another.

Isolates from the Ft. Sherman area in Panama would require the use of WR209, WR225 and WR285 as reference subspecies. Any of the three enzymes could be used to establish that an unknown is L. m. amazonensis and not the other subspecies, while MPI could be used to establish that the unknown as either L. b. panamensis or L. b. braziliensis. From this example it is evident that baseline studies on isozyme polymorphism are required to select the few taxonomically relevant enzymes to be used in a simplified identification procedure.

#### Approach

This study was designed to establish methods which the clinical laboratory technician can follow in the field to rapidly and accurately identify leishmanial isolates from U.S. military personnel. Emphasis was placed on identification of New World and Kenyan isolates in support of military operations in Central America and USAMRU-Kenya.

Isolates which are grown in any medium that can support sufficient growth of promastigotes are examined by CAE using up to 29 enzyme systems. Attempts were made to study at least 20 different isolates of each subspecies with adequate histories to obtain information on natural polymorphism. Standard enzyme profiles have been established in a previous study,<sup>11</sup> and unknown isolates were compared to these standard profiles. The enzyme profiles of each subspecies are compiled to determine which enzymes have taxonomic value.

Although the procedure for CAE identification of leishmanial isolates is simple, it should be possible to further reduce the time

and effort required to rapidly and accurately identify isolates. A simple kit designed for a particular geographic area will be assembled. This kit will contain instructions, buffers, stains, controls, and information on interpretation of results.

#### Technical Objectives

1. Determine the minimal biomass of cells needed to produce enzyme data for identification.
2. Characterize large numbers of isolates of each Leishmania to obtain information on population polymorphism which might interfere with identification.
3. Correlate data from 1 and 2 to prepare a short and accurate CAE test for identification.
4. Characterize and identify isolates from U.S. military personnel for at least 20 enzymes.
5. Concurrent with 1-4 search for possible parasite parameter/enzyme data relationships.
6. Continue to adapt additional enzyme systems to Leishmania cells searching for diagnostic enzymes.
7. Collaborate with WRAIR scientists and contractees.

#### Results

##### Isolates Identified

The isolates in this study have been characterized for the enzyme listed in Table 1. Certain isolates have been designated as WHO reference isolates, Table 2. The enzyme profiles of these enzymes have been used as standards, and the profiles of other

isolates have been compared to these reference profiles. A major objective of this study was to obtain CAE data from large numbers of isolates to obtain information on population polymorphism. The isolates which have been identified are listed in Table 3. Table 4 combines the information on geographical distribution of the isolates studies, and data on the number of isolates of each Leishmania which have been studied are included in Table 5.

In this study enzyme data from over 20 isolates of New World visceral, L. donovani chagasi and Old World visceral, L. d. donovani, have been obtained. For most of the enzymes studied (Appendix 1, Table 2) both groups are identical and monomorphic; however, for ASAT, GOT and MDH the allomorph frequency differences between the two groups of isolates are as follows:

	ASAT*		GOT*		MDH	
	Fast	Slow	Fast	Slow	Fast	Slow
<u>L.d.d.</u>	0.69	0.31	0.50	0.50	0.31	0.69
<u>L.d.c.</u>	0.00	1.00	0.00	1.00	0.00	1.00

\*ASAT and GOT might be the same enzyme.

These data suggest that if a visceral isolate has the "slow" allomorph for ASAT and MDH, it is only 20% likely that it is LD. Similar data are not yet available for separating L. infantum and L.d.c.

L. mexicana mexicana and L. m. amazonensis have distinct enzyme profiles. At present only two isolates of L. m. garnhami and two of L. m. pifanoi have been studied. No consistent differences have been noted between the profiles of L.m.a. and L.m.g. or the

profiles of L.m.m. and L.m.p.; therefore, until larger samples of LMG and LMP are studied, it is not possible to separate these pairs.

The profile of L. b. guyanensis is very similar to that of L. b. panamensis, but both profiles are quite different from the profile of L. b. braziliensis. The profiles of L.b.p. and L.b.g. differ as follows:

ACP		MDH		ME		6PGDH	
<u>L.b.p.</u>	<u>L.b.g.</u>	<u>L.b.p.</u>	<u>L.b.g.</u>	<u>L.b.p.</u>	<u>L.b.g.</u>	<u>L.b.p.</u>	<u>L.b.g.</u>
A 1.00*	A 0.00	A 0.68	A 0.00	A 0.05	A 1.00	A 0.00	A 1.00
B 0.00	B 1.00	B 0.32	B 1.00	A 0.84	B 0.00	B 0.33	B 0.00
C 1.00	C 0.00			C 0.06	C 0.00	C 0.67	C 0.00
D 0.00	D 1.00						

\*Allomorph frequencies.

Therefore, data from ACP and 6PGDH can be used to identify isolates of these two subspecies.

In summary CAE enzyme profiles have been established for L.b.p., L.b.b., L.b.g., L.m.m./L.m.p., L.m.a./L.m.g., L. m. aristedi, L. m. enriettii, L.d.d., L.d.c., L. major, L. tropica, L. aethiopica, L. hertigi hertigi and L. gerbilli.

#### Leishmania diversity

As noted in previous reports there appears to be a direct relationship between the number of isolates characterized from a particular geographical locale and the number of subspecies identified in that area. Among 83 isolates from Panama, 37 from Brazil, 50 from Colombia, and 14 from Belize there is a higher level of subspecies diversity than among isolates from other geographical

regions which have been less vigorously studied (Table 4). These supporting data on the relation between diversity and numbers of isolates examined emphasize the need to examine large numbers of isolates from all geographical areas from which leishmaniasis has been reported to obtain an accurate picture of the degree of Leishmania diversity.

Routine identification for Walter Reed Army Institute of Research (WRAIR) contractees

In addition to the WRAIR primary isolates, samples for CAE identification were received from and identified for individual WRAIR personnel and contractees. These are noted in Table 3. Certain of the identified Colombian isolates are not in the WRAIR cryobank. In each case the isolate identification was based on comparative data from previously identified WHO reference strains. In certain cases the CAE identification was made prior to initiation of other studies on the isolate and later follow up confirming identification was made during the course of studies by the contractee. It is suggested that groups involved in Leishmania research either establish CAE identification in their own laboratories or that identification confirmation be made at this laboratory prior to study. In addition CAE identification should be made a standard procedure in each project. Preliminary and confirmatory CAE identification is considered necessary, because certain isolates received at this laboratory from various sources and labeled as a particular species were identified by CAE as being other than the indicated species or as mixed cultures. Identification by CAE is too simple and accurate not to take advantage of the process.

## Short test for isolate identification

### General

Isolates of a particular subspecies have a high level of enzyme similarity (over 75%), but usually no two isolates are 100% identical. The allozyme differences among isolates are the result of natural polymorphism and can be of importance when studying biological parameters other than identification. Enzyme polymorphism can affect electrophoretic identification of Leishmania isolates. If, for example, 6PGDH were the only enzyme used for an identification, L.b.p., L.b.b., and L.b.g. could be separated even though two differently migrated bands have been observed among isolates of L.b.p. and L.b.b. (non-overlapping polymorphism). L.m.m. and L.m.a. which are also polymorphic for 6PGDH could not be separated because their polymorphism is overlapping. Then 6PGDH can identify L. braziliensis subspecies but not L. mexicana species. Enzymes chosen for a mini test identification should have either no or non-overlapping polymorphism, but ones which can separate Leishmania species and subspecies. Another consideration is the choice of enzymes which are very active (i.e. produce bands with small numbers of cells) and which are relatively simple to prepare. One monomorphic enzyme, simply prepared, with distinctly migrating bands for each Leishmania subspecies would be sufficient, unfortunately no single enzyme yet studied meets all of these requirements.

Population enzyme polymorphism is probably the most difficult parameter to determine. It requires study of many isolates (ideally 20) from the entire distributional range of the subspecies, but for

many Leishmania subspecies only a few isolates from a restricted geographical area have been studied. In this study attempts were made to reduce possible errors in biochemical identification by collecting data from many isolates with well documented (if possible already identified) histories from multiple geographical areas, Tables 3, 4 and 5. The data from the isolates already run indicate that enzyme polymorphism is either minor or not present for the enzymes GOT, GPI, GSR<sub>1</sub>, ICD, MDH, MPI and 6PGDH; furthermore, these enzymes can be used to separate most New and Old World Leishmania. These enzymes produce good activity from small numbers of cells, and buffer/strain components have been changed so each system can be preweighed, sent through the mail and requires only the addition of distilled water prior to use. Although there are eight enzymes noted, the recommended short-test for Leishmania identification will include only GPI, MPI, and 6PGDH.

The preparation of this short test for identification of Leishmania is the major objective of this study. Coincidentally, it has been established that CAE identification plus confirmation data can be obtained for the enzymes GPI, MPI, 6PGDH from about 10<sup>6</sup> cells. The identification is accurate, and the procedures as enumerated in Appendix 1 should make it possible for any laboratory with the logistical capabilities necessary to culture the parasite with minor additional equipment and chemical purchase be able to identify their isolates by CAE.

In summary most Leishmania for which enzyme profiles are available can be separated by study of three enzymes, GPI, MPI and



6PGDH. These are active enzymes for which CAE procedures have been simplified and among which only minor enzyme polymorphism has been observed. Most of these data on the short test have already been made available to and are being used by personnel in the Leishmania section at WRAIR for isolate identification.

#### Computer analysis

The CAE data which have been generated by this and other studies have been compiled in a computer program so they are readily available to personnel at WRAIR. The program for the CAE data is menu operated and IBM compatible. Data from each Leishmania subspecies are combined into a file, and each file has columns for ID number, parasite species, date, enzyme and polymorphism. This system allows simultaneous determination of numbers of enzymes used for isolate identification, allomorphs of the isolate and a comparison of the isolate's enzyme polymorphism with the polymorphisms of all other isolates of the same subspecies. In addition it facilitates the polymorphism analysis of each subspecies, as well as providing a compiled visible picture of which enzymes remain to be tested for an isolate. Disk containing these data have been forwarded to the Leishmania section at WRAIR. It was suggested by Major Lovelace that it was necessary to include the tables from these disks in the final report; therefore, I have not included them but note their availability.

#### New enzyme

One new enzyme system has been developed, GSR<sub>2</sub>. It is an isozyme with GSR<sub>1</sub>. Data from GSR<sub>2</sub> can be used to separate the

Leishmania in the complexes L. braziliensis, L. mexicana and L. donovani, Fig. 1. Unfortunately, data from not one of the enzymes listed in Table 1 can separate all Leishmania, but as noted in Appendix 1 most can be separated by study of the enzymes GPI, MPI, and 6PGDH. The CAE procedures for GSR<sub>2</sub> has been included in Appendix 1, Table 1.

Publications and reports at meetings

Kreutzer, R. D., N. Souraty and P. B. McGreevy. 1983. New World diffuse cutaneous leishmaniasis: possibly one enzyme type.

Annual meeting of Am. Soc. Trop. Med. Hyg.

McGreevy, P. B., R. D. Kreutzer, E. D. Franke, H. A. Stimson, C. N. Oster and L. D. Hendricks. 1983. Taxonomy, clinical pathology and prognosis of leishmaniasis in U. S. soldiers infected in Panama. Annual meeting Am. Soc. Trop. Med. Hyg.

Kreutzer, R. D. and N. Souraty. 1984. Accurate identification of Leishmania isolates by study of three enzymes. Annual meeting Am. Soc. Trop. Med. Hyg.

Kreutzer, R. D., N. Souraty, P. B. McGreevy and E. D. Franke. In review. A New World Leishmania which can cause either cutaneous or diffuse cutaneous leishmaniasis in human hosts. Am. J. Trop. Med. Hyg.

Chulay, J. D., C. N. Oster, P. B. McGreevy, R. D. Kreutzer and L. D. Hendricks. In review. American cutaneous leishmaniasis: clinical presentation and problems of patient management. Annals Intern. Med.

Kreutzer, R. D., N. Souraty and M. E. Semko. In review. Biochemical identities and differences among Leishmania species and subspecies. Am. J. Trop. Med. Hyg.

Table 1. Enzymes tested in this study.

<u>Enzyme</u>	<u>Enzyme Abbreviation</u>
<b>Oxidoreductases</b>	
Lactate dehydrogenases (1.1.1.27)	LDH
Malate dehydrogenase (1.1.1.37)	MDH
Malic enzyme (1.1.1.40)	ME
Isocitrate dehydrogenase (1.1.1.42)	ICD
Phosphogluconate dehydrogenase (1.1.1.44)	6PGDH
Glucose-6-phosphate dehydrogenase (1.2.1.49)	G6PDH
Gluthione reductase (1.6.4.2)	GSR <sub>1</sub> , GSR <sub>2</sub>
<b>Transferases</b>	
Glutamate-oxaloacetate transaminase (2.6.1.1)	GOT & ASAT
Glutamate-pyruvate transaminase (2.6.1.2)	ALAT
Hexokinase (2.7.1.1)	HK
6-Phosphofructokinase (2.7.1.11)	FK
Phosphoglucomutase (2.7.5.1)	PGM <sub>1</sub> , PGM <sub>2</sub>
<b>Hydrolases</b>	
Acid phosphatase (3.1.3.2)	ACP
Peptidase D (3.4.13.9)	PEPD
<b>Lyases</b>	
Fumerate hydratase (4.2.1.2)	FUM
<b>Isomerases</b>	
Mannose phosphate isomerase (5.3.1.8)	MPI
Glucose phosphate isomerase (5.3.1.9)	GPI

Table 2. WHO Reference isolates.

<u>Species</u>	<u>Reference Number</u>
<u>L. braziliensis panamensis</u> ( <u>L.b.p.</u> )	MHOM/PA/71/LS94 WR576
<u>L. b. guyanensis</u> ( <u>L.b.g.</u> )	MHOM/BR/75/M4147 WR577
<u>L. b. braziliensis</u> ( <u>L.b.b.</u> )	MHOM/BR/75/M2903 WR675
<u>L. mexicana amazonensis</u> ( <u>L.m.a.</u> )	IFLA/BR/67/PH8 WR670
	MHOM/BR/73/M2269 WR669
<u>L. m. garnhami</u> ( <u>L.m.g.</u> )	MHOM/VE/76/JAP78 WR673
<u>L. m. mexicana</u> ( <u>L.m.m.</u> )	MNYC/BZ/62/M379 WR668
	MHOM/BZ/82/BZ21 WR667
<u>L. m. pifanoi</u> ( <u>L.m.p.</u> )	MHOM/VE/57/LL167 WR671
<u>L. m. aristedesi</u> ( <u>L.m.ar.</u> )	MORY/PA/68/GML-3 WR681
<u>L. m. enriettii</u> ( <u>l.m.e.</u> )	MCAV/BR/45/L88 WR678
<u>L. donovani chagasi</u> ( <u>L.d.c.</u> )	MHOM/BR/74/M2682 WR685
<u>L. d. donovani</u> ( <u>L.d.d.</u> )	MHOM/ET/67/L82 WR684
	MHOM/IN/80/DD8 WR657
<u>L. d. infantum</u> ( <u>L.d.i.</u> )	MHOM/FR/80/LEM235 WR658
<u>L. major</u> ( <u>L.mj.</u> )	MHOM/IL/67/Jericho II WR662
	MHOM/SU/73/5-ASKH WR661
	MRHO/SU/59/NGAL P WR663
<u>L. tropica</u> ( <u>L.t.</u> )	MHOM/IQ/65/LRC-L32
	MHOM/SU/60/LRC-L39 WR683
	MHOM/SU/74/K27 WR664
<u>L. aethiopica</u> ( <u>L.ae.</u> )	MHOM/ET/72/L100 WR666
<u>L. hertigi hertigi</u> ( <u>L.h.h.</u> )	MCOE/PA/65/C8* WR679
<u>L. gerbilli</u> ( <u>L.ge</u> )	MRHO/CN/60/LUMP1575 WR683

Table 2. Continued

<u>L. tarentolae</u> ( <u>L.ta.</u> )*	ATAR/DZ/34/TARI I
<u>L. adleri</u> ( <u>L.ad.</u> )*	RLAT/KE/54/LRC-L123
<u>L. agamae</u> ( <u>L.ag.</u> )*	RAGA/IL/29/LRC-27

\*Not WHO reference isolates.

TABLE 3. CAE isolate identification.

<u>WRAIR No.</u>	<u>Category</u>	<u>Parasite Species</u>	<u>Host Species</u>	<u>Source</u>	<u>Locality</u>	<u>Other Designation</u>
271B <sup>1</sup>	OWV	LD <sup>10</sup>	Man	USAMRU-K	Kenya	LV650
307	OWV	LD	Man	USAMRU-K	Kenya	209762/400-78
310	OWV	LD	Man	USAMRU-K	Kenya	298807
312	OWV	LD/LMM-P	Man	USAMRU-K	Kenya	307024
326	OWV	LD	Man	USAMRU-K	Kenya	321827
417	NWC	LMA-G	Hamster	LAN-SHAW	Brazil	LV79, MI841, LRCL309
PJ001 <sup>2</sup>	NWV	LC	Man	P.Jackson/ Reed	Brazil	BA3
PJ002	NWV	LC	Man	P.Jackson/ Reed	Brazil	BA4
PJ003	NWV	LC	Man	P.Jackson/ Reed	Brazil	BA7
PJ004	NWV	LC	Man	P.Jackson/ Reed	Brazil	BA9
PJ005	NWV	LC	Man	P.Jackson/ Reed	Brazil	BA10
PJ006	NWV	LMJ	-	P.Jackson/ Reed	Brazil	BA11
PJ007	NWV	LC	Man	P.Jackson/ Reed	Brazil	BA12
PJ008	NWV	LC	Man	P.Jackson/ Reed	Brazil	BA13
CLO02B <sup>3</sup>	NWC	LMM-P	Man	Corredor	Colombia	MHOM/CO/84/CLO02B
CLO05B	NWC	LMM-P	Man	Corredor	Colombia	MHOM/CO/84/CLO05B
CLO18A	NWC	LMM-P	Man	Corredor	Colombia	MHOM/CO/84/CLO18A
CLO20A	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CLO20A
CLO21	NWC	LMA-G	Man	Corredor	Colombia	MHOM/CO/84/CL)21
CLO22	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/84/CLO22
CLO23	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/84/CLO23
CLO24	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CLO24
CLO26	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CLO26
CLO27	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CLO27
NO32 <sup>4</sup>	NWC	LBB	Man	Sacks	-	Torrez
NO33	NWC	LB/LMA-G	Man	Sacks	-	Broos
NO34	NWC	LBB	-	Sacks	-	WR608
NO35	NWC	LBB	-	Sacks	-	WR605
NO36	NWC	LBB	-	Sacks	-	WR604
NO37	NWC	LBB	-	Sacks	-	WR603
NO39	OWV	LD	Man	Neva	India	Mongi
NO40	OWC	LAE	Man	Neva	Ethiopia	Hulum
NO41	OWC	LAE	Man	Neva	Ethiopia	Kassahun
NO42	OWC	LAE	Man	Neva	Ethiopia	Gede
NO43	OWC	LAE	Man	Neva	Ethiopia	Degu
NO44	OWC	LAE	Man	Neva	Ethiopia	Kassaye
NO45	OWV	LD	Man	Neva	India	Sebalek
NO46	OWV	LD	Man	Neva	India	Child
NO47	NWC	LMM-P	Man	Neva	-	Morton

TABLE 3. CAE isolate identification - continued.

<u>WRAIR No.</u>	<u>Category</u>	<u>Parasite Species</u>	<u>Host Species</u>	<u>Source</u>	<u>Locality</u>	<u>Other Designation</u>
047	OWC	LT	Man	Bray	Bengal	L117RG, LRCL160, WR221B
221	OWC	LT	Man	Bray	Bengal	L117RG, LRCL160, WR047
339	OWV	LD	Man	USAMRU-K	Kenya	1336
350	OWC	LT	Man	-	Iraq	LRCL32, LV142, WR296
353	OWC	LMJ	<u>Tatera</u> sp.	Baringo	Kenya	LRCL119, LV181, LUMP1972
372	OWV	LD	Man	USAMRU-K	Kenya	L89
375	OWV	LD	Man	USAMRU-K	Kenya	IDH1908, 380544
576	OWC	LT	-	-	-	-
625	NWC	LBP	Man	WRAIR	Panama	-
626	NWC	LBP	Man	WRAIR	Panama	-
626B	NWC	LBP	Man	WRAIR	Panama	-
627	NWC	LBP	Man	WRAIR	Panama	-
637	NWC	LBP	Man	WRAIR	Panama	-
638	NWC	LBP	Man	WRAIR	Panama	-
639	NWC	LBP	Man	WRAIR	Panama	-
640	NWC	LBP	Man	WRAIR	Panama	-
641	NWC	LBP	Man	WRAIR	Panama	-
642	NWC	LBP	Man	WRAIR	Panama	-
642B	NWC	LBP	Man	WRAIR	Panama	-
643	NWC	LBP	Man	WRAIR	Panama	-
644	NWC	LBP	Man	WRAIR	Panama	-
645	NWC	LBP	Man	WRAIR	Panama	-
646	NWC	LBP	Man	WRAIR	Panama	-
654	NWC	LBP	Man	WRAIR	Panama	-
CL001B	NWC	LBP	Sandfly	Corredor	Colombia	ITRA/CO/81/CL001B
CL002C	NWC	LMM-P	Sandfly	Corredor	Colombia	MHOM/CO/84/CL002C
CL005C	NWC	LBP	Sandfly	Corredor	Colombia	MHOM/CO/84/CL005C
CL006B	NWC	LBP	Sandfly	Corredor	Colombia	ITRA/CO/81/CL006B
CL015B	NWC	LBP	Sandfly	Corredor	Colombia	ITRA/CO/84/CL015B
CL018B	NWC	LMM-P	Man	Corredor	Colombia	MHOM/CO/84/CL018B
CL031	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/84/CL031
CL033	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/84/CL033
CL035	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/84/CL035
CL038	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/84/CL038
CL039	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CL039
CL041	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/84/CL041
CL042	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CL042
CL043	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CL043
CL044	NWV	LC	Man	Corredor	Colombia	MHOM/CO/84/CL044
K030	NWC	LBG	-	Keithly	-	-
K031	NWC	LBG	-	Keithly	-	-
K032	NWC	LBG	-	Keithly	-	-
K033	NWC	LBG	-	Keithly	-	-
K034	NWC	LBG	-	Keithly	-	-
K035	NWC	LMA-G	-	Keithly	-	-
K036	NWC	LMA-G	-	Keithly	-	-

TABLE 3. CAE isolate identification - continued.

<u>WRAIR No.</u>	<u>Category</u>	<u>Parasite Species</u>	<u>Host Species</u>	<u>Source</u>	<u>Locality</u>	<u>Other Designation</u>
126	NWC	LMM-P	Man	WRAIR	Texas	WR127, ATCC30883. LV468
363	NWC	LMA-G	Man	WRAIR	Brazil	LB016C, M1287
367N	NWC	LBB	Man	WRAIR	Brazil	LTB0014, WR386
411B	OWC	LT	Man	WRAIR	Texas	
503N	VIS	LC/D	Dog #5	WRAIR	Oklahoma	WR373R
603	NWC	LBB	Man	WRAIR	Brazil	LTB564A
606	NWC	LBB	Man	WRAIR	Brazil	LTB560
609N	NWC	LBB	Man	WRAIR	Brazil	LTB559
618	NWC	LBB/BP	Man	WRAIR	Honduras	H-12
618N	NWC	LBB/BP	Man	WRAIR	Honduras	H-12
621	NWC	LBB/BP	Man	WRAIR	Honduras	See WR620
621N	NWC	LBB/BP	Man	WRAIR	Honduras	See WR620
626N	NWC	LBP	Man	WRAIR	Panama	
626BN	NWC	LBP	Man	WRAIR	Panama	
628N	NWC	LBP	Man	WRAIR	Panama	
655	NWV	LC	Man	WRAIR	-	
656	NWC	LBP	Man	WRAIR	-	
657*	OWV	LD	Man	WRAIR	India	MHOM/W/80/DD8
658*	OWV	LI	Man	WRAIR	-	LEM235
661*	OWC	LMJ	Man	WRAIR	-	5ASKH
662*	OWC	LMJ	Man	WRAIR	Israel	MHOM/IL/67/Jer11
664*	OWC	LT	Man	WRAIR	?	K27
666*	OWC	LAE	Man	WRAIR	Ethiopia	MOHM/ET/72/L10C
667*	NWC	LMM	-	WRAIR	Belize	BEL 21
668*	NWC	LMM	Nyctomys	WRAIR	Belize	MNYC/BZ/74/M379
669*	NWC	LMA	-	WRAIR	-	M2269
670*	NWC	LMA	Sandfly	WRAIR	Brazil	IFLA/BR/67/Ph8
671*	NWC	LMP	Man	WRAIR	Venezuela	MHOM/VE/57/LL1
673*	NWC	LMG	-	WRAIR	-	JAP78
675*	NWC	LBB	Man	WRAIR	Brazil	MHOM/BR/75/M2903
676*	NWC	LBP	Man	WRAIR	Panama	MHOM/PA/71/L594
677*	NWC	LBG	Man	WRAIR	Brazil	MHOM/BR/75/M414
678*		LME	-	WRAIR	-	L88
679*		LHH	Coendou	WRAIR	Panama	MCOE/PA/65/C8
681*		LMAR	-	WRAIR	-	GML3
682*		LGE	Gerbili	WRAIR	-	GERBILLI
683*	OWC	LT	Man	WRAIR	USSR	MHOM?50/60/LRC-13
684*	OWV	LD	Man	WRAIR	Ethiopia	MHOM/ET/67/L83
685*	NWC	LC	Man	WRAIR	Brazil	MHOM?BR/74/M2681
394B	NWC	LBB?	-	WRAIR	-	-
674	NWC	LMM-P	CAT	WRAIR	-	
675N	NWC	LBB	Man	WRAIR	Brazil	MHOM?BR/75/M2903
710	VIS	Lc/LI	-	WRAIR	-	
663*	OWC	LMJ	Man	WRAIR	-	
R125		?	-	WRAIR	-	
R170	OWC	LT?	-	WRAIR	-	
R300A		LT/LMA	-	WRAIR	-	
R300B		LD/LMA	-	WRAIR	-	
R335	OWC	LT?	-	WRAIR	-	
TX HAMS	NWC	LMA-G	-	WRAIR	-	



TABLE 3. CAE isolate identification - continued.

<u>WRAIR No.</u>	<u>Category</u>	<u>Parasite Species</u>	<u>Host Species</u>	<u>Source</u>	<u>Locality</u>	<u>Other Designation</u>
CLO17B	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO17B
CLO19A	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CLO19A
CLO20B	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CLO20B
CLO25	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/84/CLO25
CLO29	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO29
CLO46	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO46
CLO47	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO47
CLO48	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO48
CLO49	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO49
CLO52	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO52
CLO58	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO58
CLO60	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO60
CLO63	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/85/CLO63
CLO64	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO64
CLO66	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO66
CLO69	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO69
CLO70	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO70
CLO72	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO72
CLO73	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO73
CLO79	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO79
CLO80	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/85/CLO80
CLO82	NWC	LBP	Man	Corredor	Colombia	MHOM/CO/85/CLO82
CLO83	NWC	LBB	Man	Corredor	Colombia	MHOM/CO/85/CLO83
CLO85	NWC	LC	Man	Corredor	Colombia	MHOM/CO/85/CLO85
K038	OWC	LMJ	-	Keithly	-	
K039	NWC	LBP	-	Keithly	-	
K041	NWC	LBB	-	Keithly	-	
K042	NWC	LBP	-	Keithly	-	
K043	NWC	LBP	-	Keithly	-	
003	NWC	LBP	Man	-	Panama	Husbands
004	NWC	LBP	Man	-	Panama	Marlow
111C	NWC	LBP	Man	-	Belize	Salgado
111LN	NWC	LBP	Man	-	Belize	Salgado
132	NWC	LBP	Man	-	Panama	Tush
154	NWC	LBP	Man	-	Panama	Hernandez
176	NWC	LBP	Man	-	Panama	Watterson
179	NWC	LBP	Man	-	Panama	Legoas
211	NWC	LBP	Man	-	Panama	Fizer
232	NWC	LBP	Man	-	Panama	Barstow
241	NWC	LBP	Man	-	Panama	Martin
246	NWC	LBP	Man	-	Panama	Boynton
282	NWC	LBP	Man	-	Panama	Morrison
322	NWC	LBP	Man	-	Panama	Kingsley
360	NWC	LBP	Man	-	Panama	Clonts
390	NWC	LBP	Man	-	Brazil	Ward
442	NWC	LBP	Man	-	Panama	Bennett
446	NWC	LBP	Man	-	Panama	Burgos
470	NWC	LBP	Man	-	Panama	Belisle
475	NWC	LBP	Man	-	Panama	Varsel
486	NWC	LBP	Man	-	Panama	Reinertsen
487	NWC	LBP	Man	-	Panama	Tyus
491	NWC	LBP	Man	-	Panama	Reinertsen

TABLE 3. CAE isolate identification - continued.

WRAIR No.	Category	Parasite Species	Host Species	Source	Locality	Other Designation
492	NWC	LBP	Man	-	Panama	Reinertsen
493	NWC	LBP	Man	-	Panama	Reinertsen
505	NWC	LBP	Man	-	Panama	Reyes
525	NWC	LBP	Man	-	Panama	Dominques L1
526	NWC	LBP	Man	-	Panama	Dominques L2
539	NWC	LBP	Man	-	Panama	Carillo
530	NWC	LBP	Man	-	Panama	Schoonmaker
063	NWC	LBG	Man	-	Peru	Terborgh/Muco
294	NWC	LBB	Man	-	Brazil	Jurandi
359	NWC	LBB	Man	-	Panama	Courtwright
410	NWC	LBB	Man	-	Panama	Askew
508	NWC	LBB	Man	-	Brazil	Barbosa
524	NWC	LMA-G	Man	-	Panama	Alderman
531	NWC	LMA-G	Man	-	Panama	Castro
140	NWC	LMM-P	Man	-	Peru	UTA
381	NWC	LMM-P	Man	-	Panama	Peters
453	NWC	LMM-P	Man	-	Dominican Republic	-
457	NWC	LMM-P	Man	-	Venezuela	-
527	NWC	LMM-P	Man	-	Venezuela	Gladys, DCL
311	OWV	LD	Man	-	Kenya	David Wambua
285	NWV	LC	Man	-	Panama	Wade
317	NWV	LC	Man	-	Panama	Natuel
341	NWV	LC	Man	-	Panama	Furr
481	-	LMAR	Man	-	Panama	<u>Oryzomys</u>
528	NWC	LMP	Man	-	Venezuela	NR
316	NWC	LSP	Man	-	Panama	Wilcoxon
077	NWC	LBP	Man	USAMRU-P	Canal Zone	-
526	NWC	LBP	Man	WRAIR	Panama	Dominquez
535	NWC	LBP	Man	WRAIR	Panama	Tyus
539	NWC	LBP	Man	WRAIR	Panama	Carrillo
556	NWC	LBP	Man	WRAIR	Panama	Evans
566	NWC	LBP	Man	Evans	Panama	LS94, LV44
568	NWC	LBG	Man	Evans	Brazil	M4147, LV476
049	NWC	LBB	Man	USAMRU-P	Canal Zone	Sablan, WR242
540	NWC	LBB	Man	Evans	Belize	BZ10
541	NWC	LBB	Man	Evans	Belize	BZ14
542	NWC	LBB	Man	Evans	Belize	BZ15
543	NWC	LBB	Man	Evans	Belize	BZ17
545	NWC	LBB	Man	Evans	Belize	BZ25
557	NWC	LBB	Man	Evans	Belize	BZ16
562	NWC	LBB	Man	Evans	Brazil	LV436, M2903
348	NWC	LMM-P	Man	Neal	Costa Rica	LV191
548	NWC	LMM-P	Man	Evans	Belize	BZ18
549	NWC	LMM-P	Man	Evans	Belize	BZ26
347	NWC	LMM	<u>Nyctomys</u>	Neal	Belize	WR450, WR458, M379, LUMP1641- 1965, LV4, L11, GML92

TABLE 3. CAE isolate identification - continued.

WRAIR No.	Category	Parasite Species	Host Species	Source	Locality	Other Designation
364	NWC	LMA-G	Man	Keithly	Brazil	UISSI50492, WR384, WR421
563	NWC	LMA-G	<u>Lutzomyia</u>	Evans	Brazil	PH8, LV10
561	NW	LHH	<u>Coendou</u>	Evans	Panama	LV42
206	NWC	Lsp	Man	WRAIR	Brazil	Collier
544	NWC	Lsp	Man	Evans	Belize	Bz21
553	NWC	Lsp	Man	Keystone	Ecuador	Chest
554	NWC	Lsp	Man	Keystone	Ecuador	Face
560	OWV	LD	Man	Evans	Ethiopia	WR354, HV3, L82, LRC-L133, LV9
564	OWC	LT	Man	Evans	USSR	LRC-L39, LV357
565	OWC	LAE	Man	Evans	Ethiopia	WR298, L100, LRC-L147, LV24
547	OWC	LMJ	Man	R. Beach	Kenya	LRC-L137
551	OWC	LMJ	Rodent	R. Beach	Kenya	NLB095
552	OWC	LMJ	Sandfly	R. Beach	Kenya	NLB144
558	OWC	LMJ	Man	Hendricks	Kenya	Beach, NLB173
559	OWC	LMJ	Man	Hendricks	Kenya	Beach, NLB175
567	OWC	LMJ	Man	Evans	Israel	LRC-L137, JERICHO11, LV561
550	OWC	UNK	Sandfly	R. Beach	Israel	NLB136A
007	NWC	LBB	Man	USAMRU-P	Panama	Flores
134	NWC	LBP	Man	USAMRU-P	Panama	Acosta
336	DCL	LMM-P	Man	NIH	Dominican Republic	Cecilio
354	OWV	LD	Man	Evans	Ethiopia	
355	OWC	— LMJ	Man	?	Jericho	
357	NWC	LMA	Man	Schnur	Brazil	
409	NWC	LBG	Man	Keithly	Surinam	Davis
463	NWC	LBG	Man	Evans	Brazil	Desouza
467	NWC	LBP	Man	Tulane	Colombia	Calcedo
578	NWC	LBP	Man	WRAIR	Panama	Placenta
579	OWC	LT	?	Sacks/NIH	?	Clone 121
580	NWC	LBB	Man	Neva	?	MUCO
581	OWC	LT	Man	NIH	?	Ackerman
582	OWC	LT	?	Sacks/NIH	?	Clone 12
584	OWC	LT	?	Sacks/NIH	Afghan	Niazy
366	NWC	LBP	Man	Zeledon	Costa Rica	LB012, HSJD1
367	NWC	LBB	Man	Keithly	Brazil	LTB014, WR386
369	NWC	LMA-G	Man	Zeledon	Costa Rica	LB011, HSJD11
467	NWC	LBP	Man	Tulane	Colombia	LTB 111
472	NWC	LBB	Man	Marsden	Brazil	Aurelino
484	NWV	LC	Man	Reed	UNK	Hevania, BA7
485	NWV	LC	Man	Reed	UNK	Wilton, BA1
513	NWV	LC	Man	Reed	Brazil	Risia, BA3
514	NWV	LC	Man	Reed	Brazil	Marcos, BA11
515	NWV	LC	Man	Reed	Brazil	Elson, BA12

Table 3. CAE isolate identification - continued.

WRAIR No.	Category	Parasite Species	Host Species	Source	Locality	Other Designation
516	OL	LME	Guinea Pig	Weinstein	UNK	
517	NWV	LC	Man	Reed	Brazil	Ranilson, BA4
518	NWV	LC	Man	Reed	Brazil	Jose, BA7
519	NWV	LC	Man	Reed	Brazil	Dominqos, BA8
520	NWV	LC	Man	Reed	Brazil	Francisco, BA9
529	OL	LME	Guinea Pig	Weinstein	UNK	
585	NWC	LMA		WRAIR	Panama	WR227
586	NWC	LMJ		WRAIR	?	WR367, WR386
587	NWC	LMJ	?	WRAIR	?	WR508
BE004 <sup>6</sup>	?	LMA-G	Hamster	WRAIR	Berman	Foot
BE006	?	LMA-G	Hamster	WRAIR	Berman	Spleen
BE008	?	LMJ	?	WRAIR	Berman	173
BE009	?	LMJ	?	WRAIR	Berman	220
BE005	?	LMJ	?	WRAIR	Berman	LTB
BE010	?	LMJ	?	WRAIR	Berman	420
BE007 <sup>7</sup>	?	LMJ	?	WRAIR	Berman	15A
G001	?	LD/LC	?	Hanson		G128A
G002	?	LD/LC	?	Hanson		G128B
G003	?	LD/LC	?	Hanson		G128C
G004	?	LD/LC	?	Hanson		G128D
G005	OWV	LD	Man	Hanson	Khartoum	Parent strain, G378
G006	OWV	LD	Man	Hanson	Khartoum	Resistant, G555
G007	NWC	LBP	Man	WRAIR	Panama	Carrillo, G539A
G008	NWC	LBP	Man	WRAIR	Panama	G539B
K028	NWC	LMA-G	?	Keithly	?	CD5/84
K026	NWC	LMM-P	?	Keithly	?	CB5/85
K029	NWC	LMA-G	?	Keithly	?	CD <sub>2</sub> 5/84
K009	?	LBG	?	Keithly	?	M1142
K010	?	LBG	?	Keithly	?	M2904
K025	?	LBB	?	Keithly	?	CA
K026	?	LMM-P	?	Keithly	?	CB
	?	LBB	?	Keithly	?	M1287
K014	?	LBG	?	Keithly	?	Wild. CP
K015	?	LBG	?	Keithly	?	Clone 8-2. CX
K016	?	LBG	?	Keithly	?	Clone 8-3. CY
K017	?	LBG	?	Keithly	?	Clone 7-D. CZ
K018	?	LBG	?	Keithly	?	CAPCP
K019	?	LMM-P	?	Keithly	?	CBS <sub>1</sub> P
K020	?	LMA-G	?	Keithly	?	CCS <sub>1</sub> P
K021	?	LD?LC	?	Keithly	?	CDPC <sub>6</sub> P
K022	?	LBB	?	Keithly	?	CES <sub>5</sub> P
K023	?	LMA-G	?	Keithly	?	CFS <sub>6</sub> P
K024 <sup>8</sup>	?	LBB	?	Keithly	?	CGS <sub>1</sub> P
B001 <sup>8</sup>	OWV	LD	Man	Marr	Ethiopia	B <sub>1</sub>
B002	OWV	LD	Man	Farrell	Ethiopia	B <sub>2</sub>
B003	OWV	LD	Man	Blackwell	Ethiopia	B <sub>3</sub>
233	NWC	LMA-G	Proechimys	Lainson	Brazil	WR302, LUMP1718- 1899, LV78, M1845, LB016C, M1287

TABLE 3. CAE isolate identification - continued.

WRAIR No.	Category	Parasite Species	Host Species	Source	Locality	Other Designation
321	NWC	L?	Man	UNK	Brazil	211.497, GOTAS
363	NWC	LMA-G	Man	Lair	Brazil	
374	NWC	Lsp	Man	Beach	Surinam	Pepper Trail
405	NWC	LBB	Man	Keithly	Brazil	Ferreira. LTB082
473	NWC	LBB	Man	Marsden	Brazil	Julio
489	NWC	LMG	Man	Peters	Venezuela	JAP78, LUMP1568
508	NWC	LBB	Man	Marsden	Brazil	LTB0012
509	NWC	LMA	Man	Marsden	Brazil	Marino, B05-2
601	UNK	LD	UNK	UNK	UNK	
604	NWC	LBB	Man	UNK	Brazil	LTB558
607	NWC	LBB	Man	UNK	Barbosa	LTB12, Mucosa
608	NWC	LBB	Man	UNK	Brazil	LTB300
609	NWC	LBB	Man	UNK	Brazil	LTB559, Corte de Pedro
610	NWC	LBB	Man	UNK	Manause	
611	NWC	??	<u>Lutzomyia</u>	UNK	Brazil	
CLO02A	NWC	LMM-P	Man	Tesh	Colombia	Sanchez, Y-JS
CLO13A	NWC	LBP	Man	Tesh	Colombia	Posado, Y-BP
CLO05A	NWC	LBP	Man	Tesh	Colombia	Maturana, Y-LOM
CLO16	NWC	LBP	Man	Tesh	Colombia	Artamide, Y-LA
A0019	UNK	L?	UNK	Anthony	UNK	
A002	UNK	L?	UNK	Anthony	UNK	
A003	UNK	LMJ	UNK	Anthony	UNK	
A004	UNK	LMJ	UNK	Anthony	UNK	
A005	UNK	L?	UNK	Anthony	UNK	
A006	UNK	LMJ	UNK	Anthony	UNK	
A007	UNK	L?	UNK	Anthony	UNK	
A008	UNK	LMM	UNK	Anthony	UNK	
A009	UNK	L?	UNK	Anthony	UNK	
BE001	UNK	LMJ	UNK	Berman	UNK	Ullman. from amastigotes 508B
BE002	UNK	LMJ	UNK	Berman	UNK	Old pros. 508A
BE003	UNK	LMJ	UNK	Berman	UNK	
K001	UNK	LBG	UNK	Keithly	New World	CUMC1 7/84
K002	UNK	LBB/LD	UNK	Keithly	New World	CUMC2, 7/84
K003	UNK	LBB/LD	UNK	Keithly	Old World	CUMC3, 7/84
K004	UNK	LBG	UNK	Keithly	New World	X 9/84
K005	UNK	LBG	UNK	Keithly	New World	Y 7/84
346	NWC	LMA	<u>Orizomys</u> <u>capito</u>	Neal	Brazil	LV81, M1824

<sup>1</sup>Walter Reed isolates<sup>2</sup>P. Jackson isolates<sup>3</sup>Colombia isolates<sup>4</sup>NIH isolates<sup>5</sup>Keithly isolates<sup>6</sup>Berman isolates<sup>7</sup>Hanson isolates<sup>8</sup>Bonventre isolates<sup>9</sup>Anthony isolates<sup>10</sup>CAE identificationLMM-P - Leishmania mexicana mexicana - pifanoiLMA-G - L. m. amazonensis - garnhamiLMG - L. garnhamiLMP - L. pifanoiLBB - L. braziliensis braziliensisLBP - L. b. panamensisLBG - L. b. guyanensis

TABLE 3. CAE isolate identification - continued.

LT - L. tropica  
LMJ - L. major  
LMAR - L. m. aristidesi  
LAE - L. aethiopica  
LD - L. donovani  
LC - L. chagasi  
LI - L. infantum  
LME - L. enrietti  
LHH - L. hertigi hertigi  
LGE - L. gerbilli

Table 4. Geographical frequency distribution of isolates.

<u>Old World</u>	<u>New World</u>
Afghanistan 1-L. <u>t</u> .	Belize 7-L. <u>b</u> . <u>b</u> ., 5-L. <u>m</u> . <u>m</u> .
Algeria 1-L. <u>ta</u> .	
China 1-L. <u>ge</u> .	Brazil 2-L. <u>b</u> . <u>g</u> ., 13-L. <u>b</u> . <u>b</u> ., 10-L. <u>m</u> . <u>a</u> .
	4-L. <u>m</u> . <u>m</u> ., 1-L. <u>m</u> . <u>e</u> ., 10-L. <u>d</u> . <u>c</u> ., 1-L. <u>h</u> . <u>h</u> .
Ethiopia 1-L. <u>d</u> . <u>d</u> ., 6-L. <u>ae</u> .	Colombia 33-L. <u>b</u> . <u>p</u> ., 3-L. <u>b</u> . <u>g</u> ., 19-L. <u>b</u> . <u>b</u> .,
	2-L. <u>m</u> . <u>a</u> ., 3-L. <u>m</u> . <u>m</u> ., 5-L. <u>d</u> . <u>c</u> .
France 2-L. <u>d</u> . <u>i</u> .	Costa Rica 2-L. <u>b</u> . <u>p</u> ., 1-L. <u>m</u> . <u>a</u> .
India 5-L. <u>d</u> . <u>d</u> ., 1-L. <u>t</u> .	Dominican Republic 6-L. <u>m</u> . <u>m</u> .
Iran 1-L. <u>d</u> . <u>d</u> .	Guatemala 3-L. <u>m</u> . <u>m</u> .
Iraq 1-L. <u>t</u> .	Honduras 1-L. <u>d</u> . <u>c</u> .
Israel 3-L. <u>m</u> . <u>j</u> ., 1-L. <u>ag</u> .	Nicaragua 1-L. <u>b</u> . <u>p</u> .
Kenya 12-L. <u>d</u> . <u>d</u> ., 6-L. <u>m</u> . <u>j</u> .,	
1-L. <u>t</u> ., 1-L. <u>ad</u> .	Panama 48-L. <u>b</u> . <u>p</u> ., 4-L. <u>b</u> . <u>b</u> ., 12-L. <u>m</u> . <u>a</u> .,
	1-L. <u>m</u> . <u>m</u> ., 1-L. <u>m</u> . <u>ar</u> ., 4-L. <u>d</u> . <u>c</u> ., 2-L. <u>h</u> . <u>h</u> .
Malta 1-L. <u>t</u> .	Peru 1-L. <u>b</u> . <u>g</u> ., 1-L. <u>m</u> . <u>m</u> .
Senegal 1-L. <u>m</u> . <u>j</u> .	Suriname 1-L. <u>b</u> . <u>g</u> .
Soviet Union 2-L. <u>m</u> . <u>j</u> ., 1-L. <u>t</u> .	United States 1-L. <u>m</u> . <u>a</u> ., 1-L. <u>m</u> . <u>m</u> .
Sudan 6-L. <u>d</u> . <u>d</u> .	Venezuela 2-L. <u>m</u> . <u>a</u> ., 1-L. <u>m</u> . <u>g</u> ., 5-L. <u>m</u> . <u>m</u> .,
	2-L. <u>m</u> . <u>p</u> .

Table 5. Numbers of isolates in each subspecies or species of Leishmania tested. Note: 20 of each type should be required to produce a valid profile of the group.  
Isolates are from various laboratories.

<u>Species or Subspecies</u>	<u>Number of Isolates</u>
<u>L.g.p.</u>	104
<u>L.b.b.</u>	56
<u>L.m.m.</u>	43
<u>L.m.a.</u>	47
<u>L.d.c.</u>	27
<u>L.d.i.</u>	2
<u>L.d.d.</u>	28
<u>L.m.j.</u>	32
<u>L.t.</u>	25
<u>L.b.g.</u>	28
<u>L.m.e.</u>	2
<u>L.ae.</u>	7
<u>L.ar.</u>	1
<u>L.m.p.</u>	2
<u>L.m.g.</u>	2
<u>L.h.h.</u>	4
<u>L.ge.</u>	1
<u>L.ta.</u>	1
<u>L.ad.</u>	1
<u>L.ag.</u>	1

\*At present it is not possible to separate L.m.m. from L.m.p., L.m.a. from L.m.g., L.d.c. from L.d.i.



GSR<sub>2</sub>

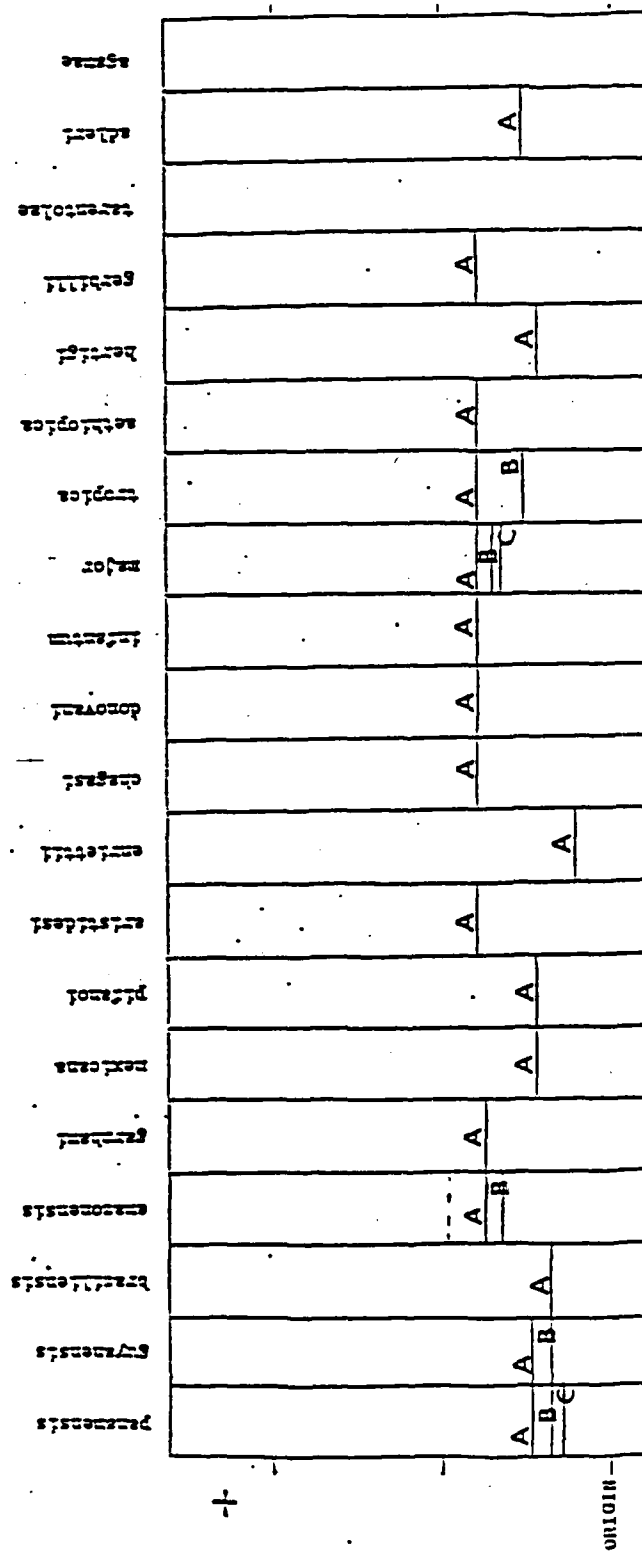


Figure 1. Diagrammatic representations of the CME patterns of GSR<sub>2</sub> for various *Leishmania*. Note: More than one band for a *Leishmania* indicates different allomorphs or natural polymorphism.

Appendix 1. Short test procedure.

Test for identification of a  $10^6$  cell biomass of Leishmania.

Data from over 400 Leishmania isolates indicate that an accurate identification of most Leishmania can be made from cellulose acetate electrophoresis (CAE) data from the enzymes glucose phosphate isomerase (GPI) EC #5.3.1.9, mannose phosphate isomerase (MPI) EC #5.3.1.8, phosphogluconate dehydrogenase (6PGDH) EC #1.1.1.44.

Materials needed:

Nobel agar

Distilled water

Chemicals - see Appendix 1, Table 1

Normal saline

Equipment needed:

Centrifuge - 1,000 g potential (cooled preferred)

Mettler type balance - 4-5 place

Nunc tubes - 2 ml

Vortex

pH meter

Stirring hotplate plus sittring bars

Stirrer

Microscope slides

Cryomarker

Microdispenser - 1-10  $\mu$ l

Pipettes - 1-100  $\mu$ l and 100-1,000  $\mu$ l

Petri dishes (100 x 45 mm)

CAE equipment (Helena Labs, Beaumont, Texas)

Titan Power Supply (Cat. No. 1500, 110V)

Zip Zone Chamber (Cat. No. 1283)

Disposable Wicks (Cat. No. 5081)

Super Z Applicator Kit (Cat. No. 4088)

- a. Applicator
- b. Sample Well Plate
- c. Aligning Base

Zip Zone Plates (Cat. No. 3023)

#### Procedure

1. Cell/medium separation and cell preparation for CAE.
  - A. About  $10^6$  cells (a visible biomass) are needed; however, visible cell masses grown on and picked off blood agar medium in petri dishes without a liquid overlay are sufficient.
  - B. Pour cells cultured in any liquid medium into centrifuging tubes and centrifuge at about 1,000 g for 5-10 min.
  - C. Pour off medium.
  - D. Add 0.5 ml normal saline to the pellet.
  - E. Transfer saline and cells to a 2 ml nunc tube and centrifuge at about 1,000 g for 5-10 min.
  - F. Remove all saline using a pipetter if necessary.
  - G. Add  $6 \mu\text{l}/10^6$  cells of buffer (14 parts distilled water: 1 part cell buffer 1 or 2 - Appendix 1, Table 1) to the cells. For larger cell pellets add buffer about 1/3 the size of the pellet.
  - H. Resuspend the cells in the buffer with a vortex.

- I. Rapidly freeze and thaw three times.
  - a. Store  $10^6$  cells/buffer at  $-70^{\circ}\text{C}$  until needed.
  - b. For larger cell masses separate lysate from the cell debris at about 1,000 g for 5-10 min., remove the lysate from the cell debris, place lysate in a 2 ml nunc tube and store at  $-70^{\circ}\text{C}$  until needed.
2. Buffer/stain preparation and conditions for CAE. See Appendix 1, Table 1. If a pH meter is available, it is recommended that cell buffer 1 be used for GPI.
  - A. Buffer preparation.
    1. Cell buffers 2-5 and reaction buffers B-G can be pre-packaged, sealed and stored at room temperature indefinitely.
    2. Combine the buffer components with the appropriate amounts of distilled water and stir until dissolved.
    3. Buffers can be prepared for any final volume, the amounts noted in the table are for preparation of one liter of each buffer.
  - B. Stain preparation.
    1. Stain components can be prepackaged, sealed and stored at  $-30^{\circ}\text{C}$  indefinitely.
    2. Combine 25 ml of the appropriate reaction buffer with 0.5 g nobel agar and bring to boiling on a stirring hotplate.
    3. Cool to  $50^{\circ}\text{C}$ .

4. While the agar is cooling, add 25 ml of the reaction buffer to the substrate/stain components and stir until dissolved. It might be necessary to use a glass rod to break up large particles which do not readily dissolve.
5. Pour the cool ( $50^{\circ}$  C) agar into the substrate stain solution and continue stirring for 5 seconds.
6. Pour the mixture into 100 x 15 mm petri dishes (5-6 dishes) and store at  $4^{\circ}$  C.
7. It is recommended that the stain plates be used as soon as possible.

### 3. Electrophoresis

#### A. Preparation of the Zip Zone chamber.

1. Add 100 ml of cell buffer to each outer well of the Zip Zone Chamber and apply a paper wick to each center rib. Remove all air bubbles.
2. If cooling is necessary, add ice cubes to the center wells. Ice should not touch the wicks.

B. Zip Zone Plates should be carefully placed in the membrane buffer (prevent trapping air bubbles in the plate) and soaked for 5-10 minutes prior to use.

C. Place 3  $\mu$ l portions in the slots of the Sample Well Plate and dilute as noted in Appendix 1, Table 1.

D. Remove Zip Zone Plates from the buffer, blot dry and place on the Aligning Base parallel to the second line from the top of the Base.

- E. Use the Applicator to transfer the lysate from the Sample Well Plates to the Zip Zone Plate.
- F. Transfer the Zip Zone Plate to the Zip Zone Chamber. The plate is applied lysate side down and application side to the negative side of the chamber. The plate should be perpendicular to the paper wicks. Place microscope slides on the plate to insure wick/plate contact.
- G. Set appropriate time and voltage.
1. After electrophoresis remove the Zip Zone Plate, blot the ends dry, cut the plate to fit the petri dish and apply it enzyme side down to the substrate/stain/agar plate. Carefully press out any air bubbles.
- H. Monitor the plates and mark the bands as they appear. Mark the plates on the plastic side with a cryomarker.
- I. After all samples have been marked, remove the plate, place it in 5% Acetic acid for 30 seconds, wash in tap water and allow to air dry.
4. Controls. To identify an isolate a comparison of the unknown to a known must be made. Control Leishmania isolates are any which have already been identified by electrophoresis and are prepared as noted above. It is recommended that only isolates designated as reference isolates by WHO be used as controls. The object of the control is to produce a band of migration so unknown isolate bands can be compared with it. Two bands

which migrate equally indicate identity. If a known isolate and an unknown produce identically migrating bands for GPI, MPI and 6PGDH, the unknown and the known are the same Leishmania.

A. Preliminary run controls in Sample Well Plate for a New World isolate.

1. L.b.b. well 3.
2. L.b.p. well 4.
3. L.m.a. well 5.
4. L.d.c. well 6.
5. Wells 1, 2, 7, 8 for unknowns or if necessary other controls.

B. Confirming run controls. After a preliminary identification has been made, a second run should be made. Each unknown should be placed next to the suspected control. This will confirm both bands migrate identically.

C. Old World isolates will require different controls.

5. Sequence of enzymes leading to identification.

A. Run GPI, Appendix 1, Figure 1.

1. Separate - L.b. complex subspecies - L.b.p., L.b.b., L.b.g.; L.d. complex subspecies - L.d.d., L.d.c., L.d.i.; L.m.m./p; L.m.a./q; L.ar.; L.t.; L.h.h.; L.ae.-L.q.e.
2. Identical band: L.b. complex - L.mj.-L.m.e.; L.d. complex; L.h.h.; L.ae.-L.ge.

- B. After GPI, run MPI Appendix 1, Figure 2.
  1. Separate (in addition to data in 5A1 above -  
L.b.b., L.ae., L.ge., L.mj., L.m.e., L.h.h.
  2. Identical band: L.d. complex, L.b.p.-L.b.b.
- C. After GPI and MPI, run 6PGDH Appendix 1, Figure 3.
  1. Separate (in addition to data in 5A1 and 5B1  
 above) - L.b.p.-L.b.b.-L.b.g.
  2. Identical band: L.d. complex.
- D. L.d. complex subspecies identification.
  1. Geographical area of isolation.
  2. Run GOT (ASAT) and MDH with L.d.d. and L.d.c.  
 controls - If both allomorphs are "slow", it is  
 35% likely that the isolate is L.d.d. or 65%  
 likely it is L.d.c. Appendix 1, Table 2.
  3. At present no reliable CAE procedures are available to  
 separate LI from LD or LC, L.m.a. from L.m.g., L.m.m.  
 from L.m.p.



#### Cell buffers:

1. 0.1 M Tris (12.11 g/l)/0.1 M Maleic acid (M-0379) (11.62 g/l)/0.01 M EDTA (ED-2SS) ( $\text{Na}_2$ ) (2.92 g/l)/0.01 M  $\text{MgCl}_2$  (M-0250) (2.03 g/l); adjust to pH 7.4 with 40% NaOH.
2. 0.1 M Tris (12.11 g/l)/0.05 M Maleic Acid (5.81 g/l)/0.01 M EDTA (2.92 g/l)/0.11 M Sodium phosphate dibasic (S-0876) (16 g/l), pH 7.4.
3. 0.2 M Phosphate buffer: 7.1 g  $\text{Na}_2\text{HPO}_4$ /3.24 g  $\text{NaH}_2\text{PO}_4$  (S-0791) in 385 mL distilled water, pH 7.0.
4. 0.29 M Tris (28.12 g/l)/0.09 M Citric acid monohydrate (C-7129) (1.89 g/l), pH 7.0.
5. 0.05 M Tris (6.06 g/l)/0.05 M  $\text{NaH}_2\text{PO}_4$  (6.0 g/l), pH 7.5.

#### Reaction buffers:

- A. 0.1 M Tris (12.11 g/l), adjust to pH 8.0 with 50% HCl.
- B. 0.06 M Tris (7.28 g/l)/0.04 M Sodium phosphate monobasic (4.72 g/l), pH 8.0.
- C. 0.25 M Tris (30.24 g/l)/0.103 M Sodium phosphate monobasic (12.34 g/l), pH 8.4.
- D. 0.1 M Tris (12.11 g/l)/0.069 M Sodium phosphate monobasic (8.21 g/l), pH 8.0.
- E. 0.018 M Sodium phosphate monobasic/0.082 M Sodium phosphate dibasic, pH 7.4.
- F. 0.1 M Tris (12.11 g/l)/0.101 M Sodium phosphate monobasic (12.12 g/l), pH 7.5.

Number of applications of the aliquot to the cellulose acetate plate:

Dilutions are made using 1 part cell buffer either buffer 1 or 2 and 14 parts distilled water.

1:1 dilution then 1X: GPI, MPI.

1X from lysate: GOT, GSR<sub>2</sub>, MDH, 6-PGDH.

\*To make 50 mL of stain (about 6 petri dishes). All chemicals from Sigma.

\*\*The membrane buffers are dilutions of 1 part cell buffer: distilled water.

\*\*\*The CA plates after electrophoresis are placed on substrate petri dishes for + 10 min. at 37°C; then blotted dry and placed on the stain petri dishes in which the bands are monitored.

<sup>a</sup>These systems require cooling during electrophoresis.

<sup>b</sup>This system requires viewing with Ultra-Violet light for the bands to be visible.

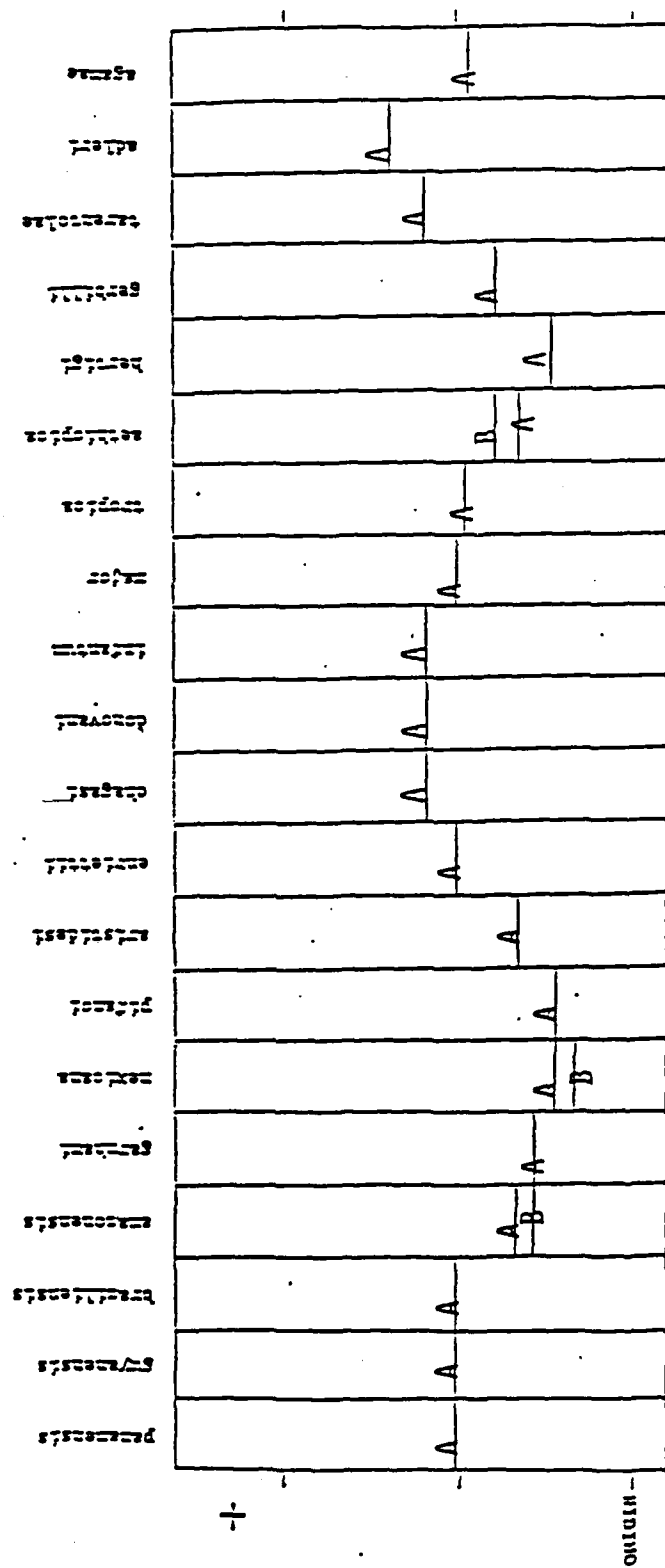
Appendix 1, Table 1. Conditions for electrophoresis and components for developing zymograms.

Enzyme	Cell Buffer	Membrane** Buffer	Voltage	Run Time (Minutes)	Reaction Buffer	Substrate/Stain Components*
GOT	1 or 2	1:14	180	15	A or D	Substrate: 100 mg L-Aspartic acid (A-9256); 75 mg $\alpha$ -Keto-glutaric acid (K-1875), readjust to pH 8.0; add 10 mg Pyridoxal-5-phosphate (P-9255); pour this mixture with agar*** by itself in petri dishes. Stain: (second petri dish) 75 mg Fast blue BB (F-3378).
GPI	1 or 2	1:14	180	15	B	20 mg Fructose-6-phosphate (F-1502); 15 mg MTT Tetrazolium (M-2128); 15 mg $\beta$ -TPN (Na salt) (N-0505); 10 mg Phenazine methosulfate (P-9625) (PMS); 60 mg EDTA; 25 units (1 flake) Glucose-6-phosphate dehydrogenase (G-6378) (Bakers yeast); 120 mg $MgCl_2$ .
GSR <sup>a</sup> <sub>2</sub>	3	1:9	200	15	C	30 mg Oxidized Glutathione (G-4376); 5 mg $\beta$ -NADH (N-8129); < 1 mg 2, 6-Dichlorophenol-indophenol (D-1878); 15 mg MTT.
MDH <sup>b</sup>	4	1:9	200	15	E	15 mg oxalacetic acetic (340-48); 15 mg $\beta$ -NADH (Na <sub>2</sub> salt).
MPI	5	1:5	160	15	F	15 mg Mannose-6-phosphate (M-8754); 10 mg $\beta$ -NADP (Na <sub>2</sub> ); 10 mg MTT; 5 mg PMS; 1 mg Glucose phosphate isomerase (P-8391); 15 units (1 flake) Glucose-6-phosphate dehydrogenase; 40 mg $MgCl_2$ .
6-PGDH <sup>a</sup>	3	1:14	180	15	B	15 mg 6-Phosphogluconic acid (P-6888) (Na <sub>3</sub> salt); 15 mg MTT; 15 mg $\beta$ -TPN; 10 mg PMS; 60 mg EDTA (Na <sub>2</sub> salt); 120 mg $MgCl_2$ .

Appendix 1, Table 2. Allomorph frequencies for ASAT and MDH in L.d.c. and L.d.d.

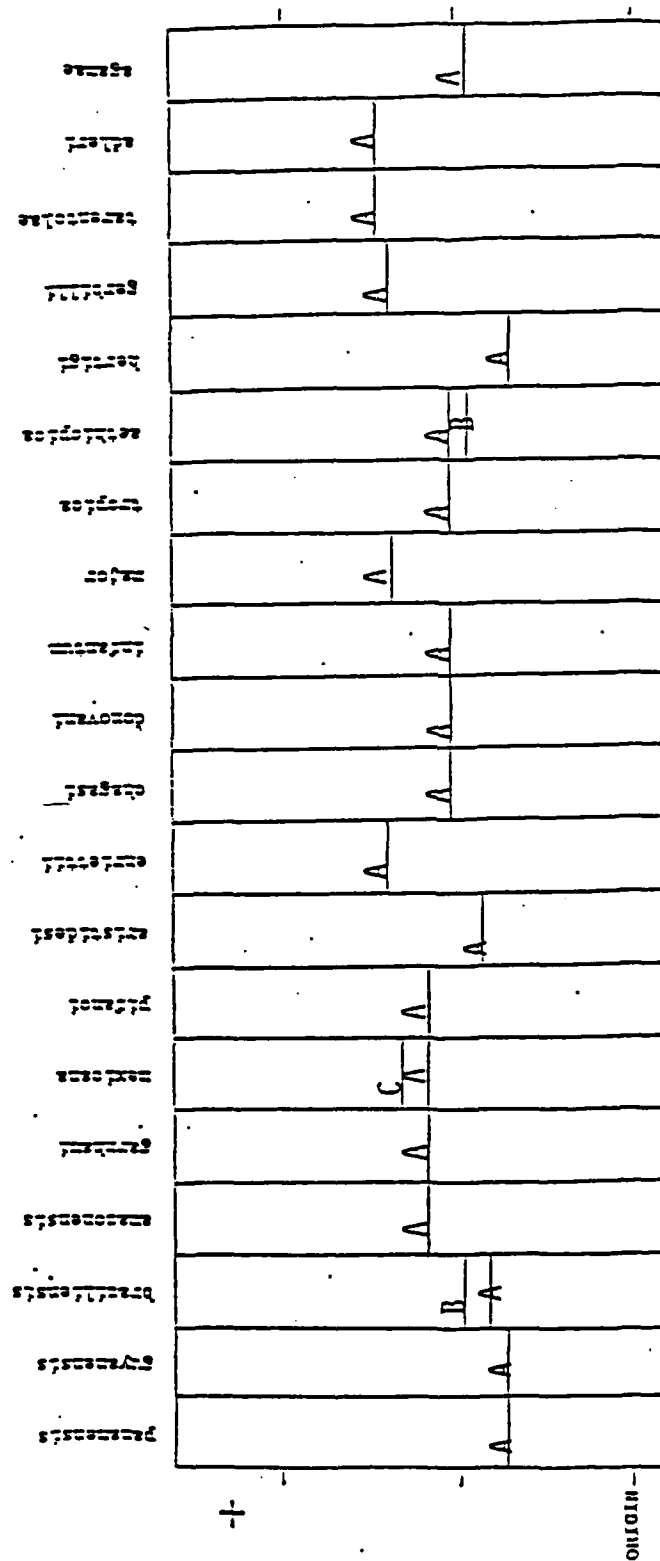
	ASAT			MDH	
	<u>L.d.c.</u>	<u>L.d.d.</u>		<u>L.d.c.</u>	<u>L.d.d.</u>
Fast	0.0	0.7	Fast	0.0	0.32
Slow	1.0	0.3	Slow	1.0	0.68

GPI



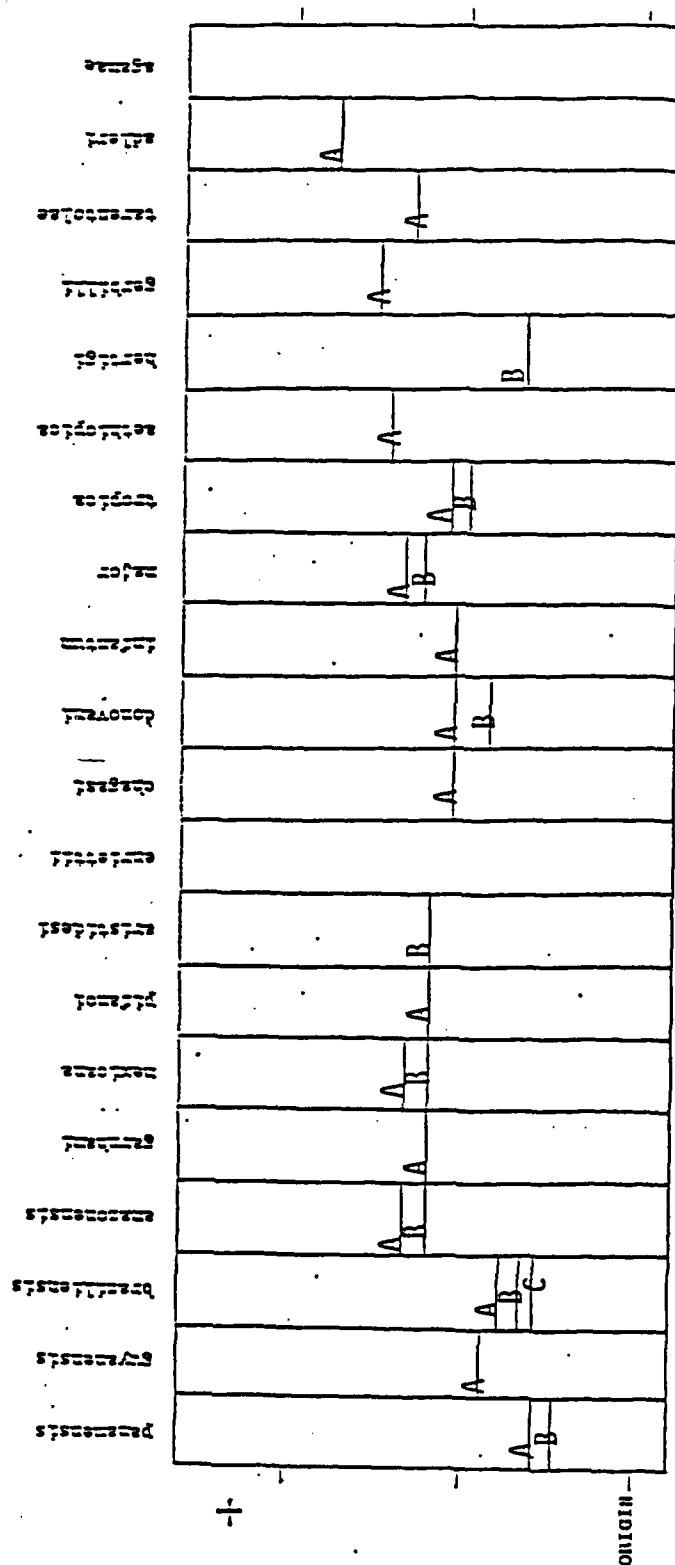
Appendix 1, Figure 1. Diagrammatic representations of the CAE patterns of GPI for various *Leishmania*. Note: More than one band for a *Leishmania* indicates different allomorphs or natural polymorphism.

MPI



Appendix 1, Figure 2. Diagrammatic representations of the CAE patterns of MPI for various Leishmania. Note: More than one band for a Leishmania indicates different allomorphs or natural polymorphism.

6PGDI



Appendix 1, Figure 3. Diagrammatic representations of the CAE patterns of 6PGDI for various *Leishmania*. Note: More than one band for a *Leishmania* indicates different allomorphs or natural polymorphism.

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